

## 3.10. Flow Cytometry-Based Proliferation Assays

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Pharmacological Inhibition of Glutaminase 1 Normalized the Metabolic State and CD4+ T Cell Response in Sjogren's Syndrome

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### Detailed protocol

cfse detection assays for proliferation

#### 1. CD4+T cell isolation

We selected 8-weeks old C57/BL6 mice and isolated the spleen of each mice in a super clean bench. Then, the spleen were ground by a Miltenyi gentle MACS Dissociator and a single-cell suspension was acquired. Then, CD4+ T cells were isolated through magnetic sorting of CD4+T cell microbeads (#19582RF, EasySep™ Mouse CD4+ T Cell Isolation Kit, Stemcells). The procedure process can be referred to the protocol in the microbeads kit.

Note: The negative selection kits are always recommended to isolated CD4+T cells. Also, a Fetal bovine serum from Gibco and RPMI 1640 are always recommended to culture the isolated CD4+T cells.

#### 2. CFSE labelling of CD4+T cells

Isolated CD4+T cells were stained with CFSE staining kits (65-0850-84, eBioscience). Here we listed the protocol of this Kits.

2.1 Prepare a single-cell suspension of cells to be labeled.

2.2 Wash cells two times with PBS to remove any serum.

2.3 Resuspend cells at 5-10x10<sup>6</sup>/mL of PBS (pre-warmed to room temperature).

2.4 Add CFSE to the desired final concentration (e.g., for a final concentration of 1 μM, add 0.2 μL of a 5 mM stock solution per mL of cells).

2.5 Mix immediately and incubate for 10 minutes at room temperature in the dark.

2.6 Stop labeling by adding 4-5 volumes of cold complete media (containing ≥10% serum) and incubate on ice for 5 minutes.

2.7 Wash cells 3 times with complete media.

2.8 Culture or transfer cells, as desired.

Note: Reconstitute one vial of CFSE to a stock concentration of 10 mM with 90 μL of anhydrous DMSO. Once reconstituted the dye should be used within 6 months, protected from light, and stored with desiccant at less than or equal to -20°C. Avoid freeze-thawing.

#### 3.[Important] CD4+T cells activation

3.1 Stained CD4+T cells were divided into a 24-well plate with the cell number of 500000-1000000 cells, in more than 1mL culture medium. It should be noted that cell density are too dense or sparse will dramatically influence the proliferation of cells after activation.

3.2 The 24-well plate was pre-coated with anti-CD3 in 100uL PBS overnight. We often retained the PBS, as we afraid that removal of PBS might also reduce the concentrations of anti-CD3. After CD4+T cells were seeded, equal concentrations of anti-CD28 should also be added. Some protocol also recommend an addition of 100 U/mL concentrations of mIL-2 for proliferation.

Note: The concentration of anti-CD3 and anti-CD28 for activation are extremely important, as the concentration of anti-CD3/28 might be varied for different brand. Our principle is to ensure each CD4+T cells should be easily assessed with enough anti-CD3/28.

3.3 Then put the plate into cell incubator for activation. The proliferation of CD4+ T cells will occur significantly after 48 hrs upon activation.

#### 4. Detection

CFSE-Labelled cells can be either detected directly (living cells) or fixed and preserved for less than 5 days, if needed.

#### EdU proliferation assays

The procedure was similar to the methods described above. We selected Beyotime EdU test kit (C0075L) in this study, the staining process can be referred to the protocol in Beyotime website. Of note, we added the EdU reagent in the cell culture medium at 24 hrs prior to harvest.

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1. Fu, J., Yu, C. and Zheng, L. (2022). 3.10. Flow Cytometry-Based Proliferation Assays. Bio-protocol Preprint. [bio-protocol.org/prep1831](https://bio-protocol.org/prep1831).
2. Fu, J., Pu, Y., Wang, B., Li, H., Yang, X., Xie, L., Shi, H., Wang, Z., Yin, J., Zhan, T., Shao, Y., Chen, C., Luo, Q., Xu, J., Zong, Z., Wei, X., Xiao, W., Yu, C. and Zheng, L. (2022). Pharmacological Inhibition of Glutaminase 1 Normalized the Metabolic State and CD4+ T Cell Response in Sjogren's Syndrome. Journal of Immunology Research 0(0). DOI: [10.1155/2022/3210200](https://doi.org/10.1155/2022/3210200)

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